Novel PET ligands for P-glycoprotein imaging

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1 Introduction

The blood-brain barrier

As early as 1885, Paul Erhlich[1] observed that some water-soluble dyes, injected into the peripheral system, did not colour the brain and cerebrospinal fluid (CSF). When injected in the subarachnoid space, however, both brain and CSF where coloured, while the peripheral system showed no colouring. A few years later, the term blood-brain barrier (BBB) was proposed by Lewandowski[2] after the observation that potassium ferrocyanide did not penetrate the brain, apparently because it did not pass a barrier between blood and brain. In 1967, Reese and Karnovsky[3] suggested that the BBB was located structurally at the level of the endothelial cells, as microscopic images showed that horseradish peroxidase did not diffuse past the tight junctions of these cells.

The function of the BBB is to protect the brain from potentially toxic exogenous compounds by preventing their entry into the brain. Apart from a protective influx barrier, the BBB also is equipped with several efflux transport mechanisms to remove compounds from the brain into the blood. One of those efflux transporters is P-glycoprotein (P-gp, where P stands for permeability), which is also known as ATP binding-cassette subfamily B member 1 (ABCB1), cluster of differentiation 243 (CD243), and multidrug resistance protein 1 (MDR1)[4]. P-gp is a 170 kDa transmembrane glycoprotein, which is ATP dependent and located at the luminal side of the brain capillary cells[5-7]. The main function of P-gp is to transport exogenous or toxic compounds from the brain back into the blood stream. In this way P-gp helps to reduce unwanted side effects of drugs by preventing their entry into the brain (e.g. non-brain penetrating antihistamines). On the other hand, it also limits penetration of certain central nervous system (CNS) drugs into the brain. In addition, chemotherapeutic agents are known to be substrates for P-gp.

After discovering that the BBB is located at the level of the endothelial cells, there has been steady progress in the understanding of P-gp function. A list of major discoveries related to P-gp is given in Table 1.

Table 1: List of major discoveries related to P-gp[11]

1968	Isolation of MDR Chinese Hamster cell lines[12].
1973	Demonstration of inhibition of drug efflux causing increased drug accumulation[13].
1974	Evidence of drug resistance being energy dependant[14].
1976	Demonstration of P-gp expression in MDR cell lines[15].
1979	First purification of P-gp[16].
1979	Isolation of MDR human cancer cell lines[17].
1981	Demonstration that verapamil can reverse MDR[8].
1984	Quinidine and related compounds inhibit the efflux of vincristine in tumor cells[19].
1985	Detection of P-gp in clinical samples[20].
1985	Definition of ABC cassette superfamily[21].
1986	Cloning of full-length MDR1 gene and demonstrating its homology to bacterial ATP dependant transporters[22].
1986	Demonstration of drug binding by membranes that contain P-gp[23].
1988	Demonstration of P-gp mediated drug transport in isolated vehicles[24].
1989	Discovery of P-gp expression in brain capilaries[25].
1990	Detection of P-gp isoforms by gene specific antibodies[26].
1992	Demonstration that P-gp is directly involved in the BBB[27].
1994	Demonstration that the MDR1 knock-out mouse has BBB defects and alterations in drug pharmacokinetics[28].
1997	First report on [^{99m}Tc]sestamibi uptake in tumours, indicating its use as a surrogate SPECT (single photon emission computed tomography) tracer of P-gp function[29].
1998	First report on [^{11}C]verapamil as a PET (positron emission tomography) P-gp tracer[30].
2012	Elucidation of the crystal structure of P-gp[31].

The role of P-gp in epilepsy

Epilepsy is a neurological disease that is characterised by seizures of various magnitudes. These seizures are the result of hyperactivity of cortical neurons in the brain. To date there is no cure for epilepsy, alltough, medication or surgery might control the seizures. In the majority of patients suffering from epilepsy (around 70%[10]), seizures can be suppressed by drugs. Nevertheless, a substantial number of patients (about 30%[10]) becomes resistant to medication. In other words, these patients develop pharmacoresistance, which is defined by the international league against epilepsy (ILAE) as “The failure of a patient’s seizures to respond to at least two antiepileptic medications that are appropriately chosen and used for an adequate period”.

Most anti-epileptic drugs are also moderate substrates for P-gp and it has been postulated that P-gp function plays an important role in pharmacoresistance in epilepsy[8]. In addition, it has been suggested that development of pharmacoresistance after prolonged treatment may be due to overexpression of P-gp[8]. This altered expression or function of multidrug transporters in the brain was first described by Remy and Beck[9] in 2006. Next, Löscher and Potschka[10] suggested that overexpression of P-gp may indeed be a direct result of repeated administration of anti-epileptic drugs.

PET tracers of P-gp function

At present, several PET tracers are available to assess P-gp functionality. These tracers are all P-gp substrates, e.g. [^{11}C]verapamil[35], [^{11}C]desmethyl loperamide[36] and [^{11}C]laniquidar[37]. As P-gp substrates, they are particularly useful for imaging downregulation of P-gp, as reduced P-gp function will result in an increased signal. In contrast, substrate tracers are inadequate to image overexpression of P-gp, as the already low PET signal will be reduced even further, resulting in signal to noise ratios that are too low for reliable measurements. To assess upregulation of P-gp, a tracer that binds to P-gp itself, without being transported, would be

ideal. Alternatively, a weaker P-gp substrate could be used as PET tracer, as such a tracer would lead to a relatively high baseline PET signal and, therefore, a reduction in signal due to increased P-gp expression would still be detectable.

Aim of the thesis

The overall aim of this thesis was to develop new PET tracers for imaging P-gp distribution and expression. To this end, three different approaches of research were envisioned.

First, it has previously been shown that [^{11}C]D617, a metabolite of (*R*)-[^{11}C]verapamil, may account for up to 30% of the PET signal in the human brain, 30 minutes after injection[35], which is a major disadvantage of (*R*)-[^{11}C]verapamil. In addition, Lubberink et al[39] also indicated that kinetics of [^{11}C]D617 were similar to those of (*R*)-[^{11}C]verapamil. In other words, [^{11}C]D617 may actually be a better P-gp tracer than (*R*)-[^{11}C]verapamil, as by definition it should have less metabolites. Therefore the first goal was to synthesize [^{11}C]D617 and assess whether it could be used as a P-gp tracer.

The second approach focussed on the development of a weak P-gp substrate PET tracer and [^{11}C]phenytoin was selected as the first candidate. As phenytoin is a registered drug and labelling with carbon-11 would not alter its chemical structure, no toxicology studies would be needed, thereby facilitating translation to human applications. Although the synthesis of [^{11}C]phenytoin has previously been described by Roeda et al[40], the synthetic pathway was tedious and molar activity was low. Therefore an alternative synthesis of [^{11}C]phenytoin should be investigated. Clearly there are several weak P-gp substrates and [^{11}C]quinidine was selected as an alternative P-gp substrate tracer.

The most important approach was to identify a potential PET tracer that binds to P-gp at tracer levels, making it possible to image P-gp expression rather than function. At present, several P-gp inhibitors could be used as basis for such a P-gp expression tracer. However, experience with existing examples, such as [^{11}C]laniquidar[37] and [^{11}C]tariquidar[38], have been

disappointing, as they appear to act as P-gp substrates at tracer levels. Therefore, for developing a true P-gp expression tracer, another class of P-gp inhibitors needs to be used as starting point and, to this end, labelling of isatin-5-(4-methoxyphenyl)-3-thiosemicarbazones, published by Hall et al[41], was selected.

Outline of the thesis

Chapter 2 provides an overview of existing carbon-11 labelled PET tracers for visualizing P-gp function, showing their benefits and flaws.

Chapter 3 describes the synthesis of desmethyl D617 and the radiosynthesis of [^{11}C]D617 together with its *in vivo* evaluation as a P-gp PET tracer in rats.

Chapter 4 describes the synthesis of [^{11}C]phenytoin and it's *in vivo* evaluation in rats.

Chapter 5 shows the metabolism and cerebral kinetics of [^{11}C]quinidine and [^{11}C]laniquidar in both control rats, and rats with spontaneous recurrent seizures before and after tariquidar.

Chapter 6 describes the synthesis of both [^{18}F]-(4'-fluoroisatin)-4-4'-methoxyphenyl)-3-thiosemicarbazone and [^{18}F]-(6'-fluoroisatin)-4-4'-methoxyphenyl)-3-thiosemicarbazone, together with the biodistribution and metabolic profile of the first in rats and P-glycoprotein knock-out mice.

Chapter 7 provides a short summary and discussion of the studies described in this thesis, together with some future perspectives for further research in P-gp PET tracer development.

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